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REVIEW ARTICLE

Insects as a potential source of chitin and chitosan: Physicochemical, morphological and structural characterization. -A review

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ABSTRACT

Insects are mega-diverse species. Structurally, insects are composed of the polysaccharide known as chitin and its deacetylated derivative, chitosan. Actually, exist few studies regarding to the physicochemical and structural characterization of these biopolymers in the main insect orders. The present study shows a review of chitin and chitosan obtained from insect sources; it was carried out on the similarities and differences between these biopolymers and those obtained from conventional sources. X-ray diffraction, infrared spectroscopy with Fourier transform, and thermogravimetric analysis are presented which are important to determine how the structural, morphological and physicochemical properties of chitin and chitosan are affected depending on the source taxonomy of insects. The main techniques used for the isolation and the yields obtained are shown. Future research will be conducted to expand chitin and chitosan applications from insect in areas as diverse as food, biotechnology and biomedicine, emphasizing that insects can represent a potential raw material.

Keywords: Biopolymeric materials; Physicochemical characterization; Insect order; Insect species; Chitin yield

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INTRODUCTION

Chitin is the second most abundant polysaccharide in nature after cellulose and is a structural part of insects, crustaceans, fungi, yeasts and algae (Velásquez, 2003; Xia et al., 2011). Chitosan (obtained by the deacetylation of chitin) constitutes the most important derivative of chitin, being a natural cationic polysaccharide composed of units of β-[1-4]-D-glucosamine and units of N-acetyl-β-[1-4]-D-glucosamine. Unlike chitin, chitosan is soluble in dilute acid solutions, which increases its applicability (Jia and Xu, 2001). Chitosan has many applications in different research areas due to its multiple properties such as biodegradability, biocompatibility and non-toxicity, in addition to its biological properties such as antimicrobial, antitumor, antioxidant, hypocholesterolemic and wound healing activity (Zheng and Zhu, 2003; Hou et al., 2011; Zhang et al., 2012; Panith et al., 2016). These attributes make it the appropriate candidate for multiple applications in areas as diverse as agriculture, and in the food and biomedical industry (Bekhit et al., 2015; Gonçalves et al., 2015; Jeyakumari et al., 2016). However, the application of chitosan is determined by its properties, which include the degree of deacetylation, molecular weight, crystallinity, purity and morphological structure (Aranaz et al., 2009; Hamed et al., 2016; Sacco et al., 2018).

The main commercial source of chitosan is shrimp and crab exoskeletons from fish and food industry waste (Yang et al., 2000; Zhang et al., 2000; Younes and Rinaudo, 2015). Anually, 8×10^6 ton of waste are produced, of which 40% are exoskeletons that contain 15 to 40% chitin (Hahn et al., 2020). However, one limitation is its low availability due to seasonal variations, coupled to its high demand due to its versatile applicability, has driven the search for alternative sustainable sources of this biopolymer, one of them are insects, which present a great unconventional potential source. The second source of chitin are fungi whose content is from 1 to 15% of the mass of cell walls; however, they are not an industrial source of chitin (Hahn et al., 2020).

Insects are another promising and sustainable source of chitin and chitososan (Hahn et al., 2020).

These are the most abundant living organisms on the planet and include between 6 and 10 million species (Luo et al., 2019). There are cosmopolitan insects of various orders, and some are considered pests, because they cause damages to human interests (crops, warehouses, production, aesthetics, comfort, among others) (Sinha and Watters, 1985). These insects are characterized by their wide distribution and polyphagia; however, in this particular case, these pest insects are being eliminated through the use of chemical, physical and biological treatments without granting them added value (Chernin and Chet, 2002). On the other hand, there are also insects whose main advantage is their artificial breeding, such as bees, silkworm, among others (Nemtsev et al., 2004), and due to their high reproductive rate, they are not affected by seasonality (Hahn et al., 2020).

The soft internal tissue of insects is covered by the exoskeleton, which is rich in chitin (da Silva Lucas et al., 2020; Hahn et al., 2020). Insects contain 30-45% protein, 25-40% lipid, and 10-15% chitin (Mohan et al., 2020), although they may contain more than 40% chitin in their exoskeleton (Khayrova et al., 2021). Chitin content varies with the development of the insect life cycle (Mohan et al., 2020). In addition, insects have less inorganic material (less than 10%) than shells of crustaceans (30 to 50%) (Khayrova et al., 2021), so chemical extraction procedures are less severe on insects (Zainol Abidin et al, 2020), for which it is possible to obtain chitin only with deproteinization (da Silva Lucas et al., 2020). In relation to proteins, insects have an average of 60% of these compounds (proteins), which are highly digestible and have an adequate amino acid profile (Zamudio-Flores et al., 2019). Studies on protein isolation from insect sources are scarce compared to studies on protein isolation from other plant or animal sources. For example, Nongonierma and FitzGerald (2017) reported a protein isolate obtained from the silkworm (*Bombyx mori*) which presented 80% solubility. In a recent study, protein isolate (82% protein) from the coleopteran *Alphitobius diaperinus* was obtained, which increased the amino acid content in the blood 2 h after consumption in humans, similar to that provided by whey protein isolates and soy (Vangsoe et al., 2018). Some insects, such as the black soldier fly (*Hermetia illucens*) have been used for the production of protein flour, which is considered a high-quality protein source and its chitin has also been isolated (Hahn et al., 2019) and obtained its chitosan (Khayrova et al., 2019).

Chitin and chitosan extracted from insects may have different physicochemical properties than those extracted from shrimp and crabs, so they may have different applications (Brigode et al., 2020). However, in a recent study it was reported that these biopolymers obtained from the house cricket (*Brachytrupes portentosus*) have the same degrees of acetylation and deacetylation as those obtained from shrimp (Ibiyote et al., 2018). In addition to the above, recently Saenz-Mendoza et al. (2020) reported that after performing FTIR and X-ray diffraction analysis, no significant differences were observed between films made from chitosans obtained from *Brachystola magna* and *Tenebrio molitor* compared to commercial chitosan films of different molecular weights. Similarly, Shin et al.

(2018) using the afore mentioned techniques (FTIR and X-ray diffraction), did not observe structural differences between insect chitosans obtained from *Tenebrio molitor* and *Allomyrina dichotoma* when compared with commercial chitosans.

Despite their properties, insectile chitosans have not been sufficiently investigated (Shin et al., 2019). The use of biomass obtained from natural waste from harmful insects could provide global benefits (Mohan et al., 2020). Extensive reviews have now been published on insects that may be an important source of chitin and chitosan. To cite a few studies, Khayrova et al. (2020) reported more than 10 insect species, of which the highest yields of chitin and chitosan, respectively, were obtained in *Hermetia illucens* (80 and 43%), *Bombyx mori* (20, 80%) and *Apis mellifera* (23 and 24). %); while Mohan et al. (2020) reported about 50 species, among which *Bombyx mori* (50%, ND), *Apis mellifera* (40 and 25%), *Cryptotympana atrata* (60 and ND) stand out. Recently, Khayrova et al. (2021) reported 12 species, with the highest yields in these biopolymers being the species of *Apis mellifera* (23 and 24%), *Bombyx mori* (20 and 80%), *Hermetia illucens* (87 and 43%) and *Neotibicen linnei* (36% and ND). Likewise, Zainol Abidin et al. (2020) presented a review of more than 80 species of crustaceans, molluscs and insects with an important content of chitin and chitosan; while Hahn et al. (2020) reported more than 50 species, among which the *Apis mellifera* insects (51-77%), *Hermetia illucens* (46%) and *Catharsius molossus* (24%) stand out for their high chitin yield. However, previous studies have shown that chitin and chitosan physicochemical properties will depend on the method and obtaining source (Paulino et al., 2006; Kaya et al., 2016; Marei et al., 2016). For this reason, this review has the objective of showing the physicochemical, morphological and structural properties of various studies focused on the evaluation of chitin and chitosan isolated from insects, in addition to illustrating the role played by some variables such as taxonomy, genus, development status and different segments of the insect's body in these biopolymers' properties. Additionally, the main applied methodologies for obtaining both biopolymers will be showed, this in order to provide information and suggestions to serve future research in the use and exploitation of insect chitins and chitosan.

CHITIN AND CHITOSAN COMMERCIAL PRODUCTION

Nowadays, the industrial production of chitin and chitosan comes from the waste of the food industry and fisheries such as crab shells and shrimp exoskeletons, mainly. According to the literature, approximately 150,000 ton, 25,000 ton and 85,000 ton of shrimp, lobster and crab, respectively, are processed annually in the USA, so that if all these wastes were used, more than 15,000 ton of chitin could be produced (Mathur and Narang, 1990). It has been reported that in order to obtain 1 kg of chitosan from shrimp husks (70% deacetylation) 6.3 kg of HCl approximately are required as well as 1.8 kg of NaOH (Kim, 2015). Commercially chitin and chitosan are produced mainly in countries such as Norway, Poland, India, Japan, and Australia (Parada et al., 2004). It has also been informed that the price of chitosan is 7.5 USD per 10 g (Kumar, 2000). Chitin and chitosan have been highly demanded in the food and non-food industry; however, their extraction is limited since they are derived from seasonal raw materials (crustacean waste) (Becerra-Jiménez et al., 2011) so the search for new alternative sources such as insects could be of great commercial interest.

INSECTS BIODIVERSITY AND GENERALITIES

Insects are one of the most diverse and abundant arthropods on earth, and although the number of species is unknown, it is estimated that 5.5 million species exist (Siripatrawan and Harte, 2010). Many insect species are of great commercial interest for humans due to their multiple uses as food (entomophagy), medicine (entomotherapy), and for the beneficial tasks related to agroecosystems, such as decomposers and biological control agents (parasitoids and predators) (Guzmán-Mendoza et al., 2016). However, other insects are considered destructive because they damage crops, stored grains, furniture, wood, among others (Sinha and Watters, 1985; De Lima, 1987; Su and Scheffrahn, 2000; Tian et al., 2004; Sarwar, 2015); bringing great economic costs.

Considering that, the problem in crops is originated when insect populations increase massively due to the availability of resources, causing damage to seeds, roots, stems, leaves, flowers, shoots and crops fruits (Mitsuhashi, 2010; Van Huis et al., 2013; Sarwar, 2015). Previously, several insect pests of different genus and species have been reported, which can present high population densities in different crops (Ramírez-Salinas and Castro-Ramírez, 2000; Coto and Saunders, 2004; Serra and Trumper, 2006). For instance, grasshoppers (Orthoptera) are common pest that may cause great damages since they eat a great variety of plants (Sirimungkararat et al., 2010; Yoshizawa and Lienhard, 2016). Some strategies for its control are chemical, biological, physical and cultural methods; however, when dying they become a serious environmental problem due to their accumulation and pollution of some of these like the chemicals (De Lima, 1987; Kaya et al., 2015; Sarwar, 2015; DiTomaso et al., 2017). For this reason, it would be important to take full advantage of the insects, for

example, considering them as a source of chitin. Under this perspective, environmental damage will decrease through the recycling of waste, which may be of great economic interest and significantly contributing to the supply of chitin and chitosan in the commercial market. Previous research suggests that pest insects can be exploited in such a way that their population can be regulated, constituting an useful resource for human populations as a source of food or biopolymers for industrial use (Cerritos and Rojas-García, 2012).

From another perspective, in the literature it has been mentioned that the artificial breeding of certain insect species could supply a large amount of raw material for the industrial processing of chitin, such as bees (*Apis mellifera*, Hymenoptera: Apidae) and silk worms (*Bombyx mori*; Lepidoptera: Bombycidae) (Nemtsev et al., 2004; Paulino et al., 2006). It is also important to emphasize that another advantage is that some insects' breeding methods are practical and low-cost (Sirimungkararat et al., 2010; Van Huis et al., 2013). On the other hand, the use of insects that are by-products or wastes from industrial processes has also been reported as an opportunity, for example, B. mori pupae are feasible to commercialize due to their availability and low price, since they are byproducts of the silk industry (Zhang et al., 2000). In the same way, the waste of the insect *Catharsius molossus* (Coleoptera: Scarabaeidae) from the extraction of the active ingredient for the treatment of benign prostatic hyperplasia (a polypeptide), present a great opportunity in its use, since these residues affect the environment (Ma et al., 2015). Despite that, as far as we know, the economic impact that insects as chitin and chitosan source may represent, has not been reported in detail yet.

Insects general characteristics include a chitin exoskeleton, a body divided in three regions (head, thorax and abdomen), three parts of articulated feet, composed eyes and a pair of antennas. On the other side, according to the insect's specie, two types of metamorphosis are distinguished: simple and complete. Complete metamorphosis insects (holometabolous), go through four stages [egg, larvae, chrysalis (pupae) and adult], and in the simple metamorphosis (hemimetabolous), the insect goes through three stages (egg, nymph and adult) (Mitra, 2013). Insects are divided in 30 orders regarding to the differences between the wings characters, mouth parts, legs and metamorphosis (Yoshizawa and Lienhard, 2016). Recently five orders have been studied in relation with the extraction and physicochemical characterization of chitin and chitosan, which will be described below:

Blattaria (Blattodea): Contains more than 4,600 species and are known as cockroaches (Faúndez and Carvajal, 2011). They are hemimetabolous and are found on soil and vegetation.

Coleoptera: Are known as beetles and is the order with the greatest amount of species (387,000) (Stork, 2018). They are hemimetabolous and are found in all type of habitats.

Hymenoptera: To this order belong bees, wasps, bumblebees, and ants; and it is estimated that there are 116,861 species (Zhang, 2011). They are holometabolous and are usually found in soil and vegetation.

Lepidoptera: They are holometabolous insects and are known as butterflies and moths. It is estimated that there are 157,000 species (Condamine et al., 2016). They are commonly found on vegetations.

Orthoptera: Contains about 24,276 species (Zhang, 2011). They are hemimetabolous and are commonly known as locusts, crickets and grasshoppers. They are usually located in soils and vegetations.

CHITIN BIOSYNTHESIS IN INSECTS

The chitin biosynthesis process includes an ordered sequence of complex cellular reactions which may initiate in three different ways: 1. From trehalose (disaccharide present in the hemolymph of insects); 2. Glycogen (glycogen of the fatty body); 3. N-acetylglucosamine (recycling) (Muthukrishnan et al., 2012). When trehalose initiates, its own hydrolysis is implied, glucose phosphorylation, transmutation to form phosphorylated fructose, amination, acetylation and its conversion to an amino-sugar phosphate and acetylated nucleotide, formation of UDO-N- acetyl glucosamine, and finally chitin synthesis through then enzyme chitin synthetase (Fig. 1) (Tharanathan and Kittur, 2003; Muthukrishnan et al., 2012). When the synthesis starts from glycogen, the glycogen phosphorylase enzyme intervenes, in which the glucose-1-phosphate produced by this reaction becomes trehalose, which is released into the hemolymph. On the other hand, it has been reported that chitin is also synthesized (it is recycled with the help of chitinolytic enzymes) as portions of the old endo-cuticle, peritrophic matrix and trachea are reabsorbed (Muthukrishnan et al., 2012).

CHITIN ISOLATION METHODS

The exoskeleton of the insect has chitin, minerals and proteins as its main components. It also contains fat and pigments (melanin) in small quantities (Finke, 2007; Draczynski, 2008). The method used at the industrial level for obtaining chitin consists of a deproteinization

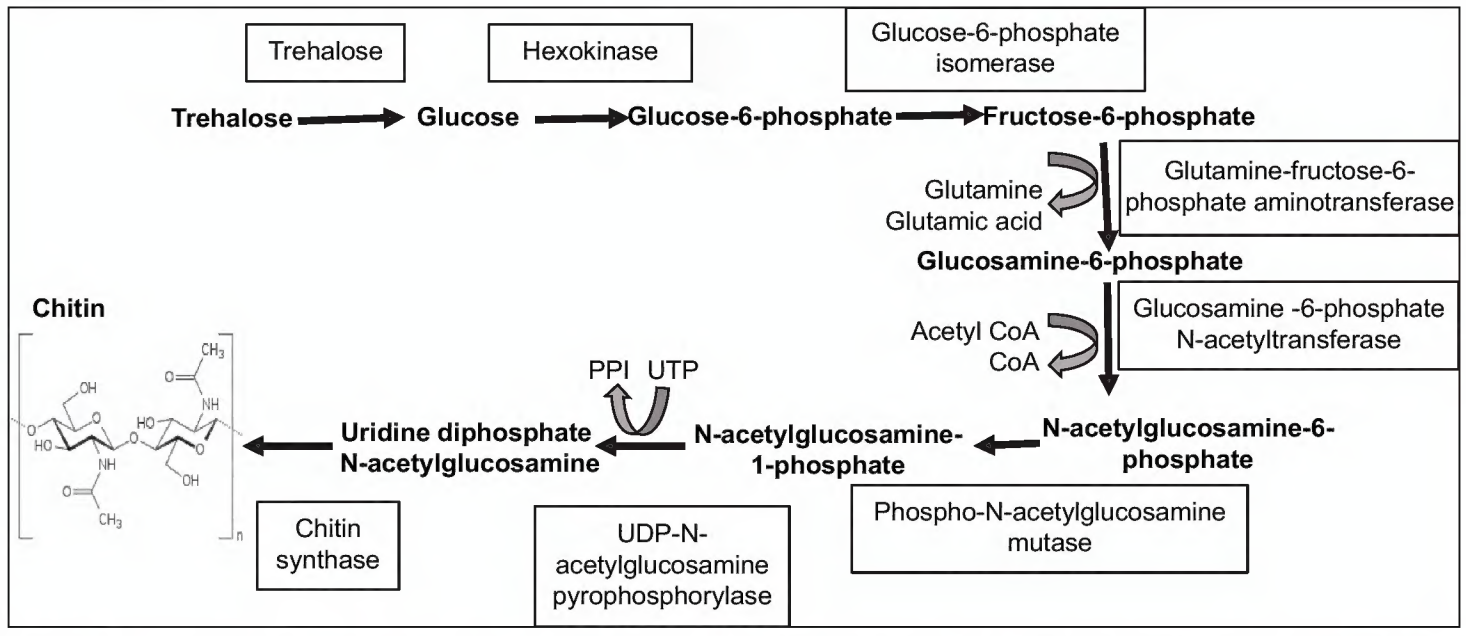

Fig 1. Chitin biosynthesis in insects from trehalose. This figure describes the pathway for chitin biosynthesis from trehalose, and the enzymes responsible for the process.

and demineralization (El Knidri et al., 2018). In some cases, decoloration steps are also included, using solvent extraction or chemical oxidation of the remaining pigments (Synowiecki and Al-Khateeb, 2003). In the stage of demineralization several reagents are used such as HCl, HNO3, H2SO3, CH3COOH or HCOOH to remove inorganic material which is linked to chitin, however HCl is the chemical reagent most frequently used. In extensive research on the extraction of chitin and chitosan in various maritime sources, it has been suggested the importance of adapting the conditions of the treatments used according to the production source, this is due to the differences in the ultra-structure of the initial material (Younes and Rinaudo, 2015). In this sense, it is important to previously evaluate the insect's composition because the mineral content varies depending on the insect order, size, sex, stage of development, season and subsequent conditions of slaughter; in which, elements such as N_2, K^{+1}, Mg^{+2}, Na^{+1}, Fe^{+3} and Ca^{+2} have been observed (Studier and Sevick, 1992). A determination in the ashes content allows to identify the amount of minerals present in the insect. In previous studies it has been observed ashes contents significantly lowers in the insects *Brachytrupes portentosus* (Orthoptera: Gryllidae), 6.8%; *Syntermes* soldiers (Blattodea: Termitidae), 4.2%; *Macrotermes bellicosus* (Isoptera: Termitidae), 5.7%; and in *Brachytrupes* spp. (Orthoptera: Gryllidae), 59.1%; compared with other crustaceans' sources (shrimp shells, 52.6%; and crab, 59.1%) (Hamdi et al., 2017; Akullo et al., 2018; Ibitoye et al., 2018). Oduor et al. (2008) determined a lower content of ashes in larvae of the insect *Calliphora erythrocephala* (Diptera: Calliphoridae) (<1%) than in the crustaceans of crab, lobster and shrimp (26.3-45.2%), for which no demineralization treatment was required. The authors attribute that this variation was due to a possible difference of the insects, since the crustaceans contain a high content of calcium carbonate in their structure. Liu et al. (2012) have suggested that due to the low content of inorganic materials in insects, moderate conditions of acid treatments can be used in comparison with crustaceans, so adjusting the treatment according to the insect raw material may imply a demineralization treatment at a lower cost.

Regarding to the procedures reported in the literature for the treatment of demineralization in insects, it can be observed that there is no uniformity in the conditions used, with concentrations between 1 and 4 M being reported for 0.3 and 24 h at temperatures varying from room temperature to 100 °C. In general, they are similar to what was reported for the Arachnida and Malacostraca classes (concentration = 0.55-4 M; time = 1-2 h; temperature = room temperature-100 °C). Table 1and 2 summarize the conditions used for the demineralization and deproteinization stage in different orders and species of the Insecta, Arachnida and Malacostraca classes.

In terms of the applicability for chitin and chitosan, as well as the uses related to human consumption (food applications) and in biomedical applications, the quality or purity that they present is important, which means that these biopolymers should present a low residual content of ash, moisture and proteins (Aranaz et al., 2009). With respect to the above, a residual ash content of 1% is of high quality (Nessa et al., 2010). In previous studies, after isolating chitin, residual contents of 1% and 2% have been observed, for *B. portentosus* and *Holitrichia parallela* (Coleoptera: Melolonthidae), respectively (Ibitoye et al., 2018; Liu et al., 2012). These differences could possibly be attributed to the content of minerals in the source, particle size and treatment conditions (acid concentration, temperature, time and solute/solvent ratio) (Poeloengasih et al., 2010; Younes and Rinaudo, 2015). According to the nature of the insects, most have a considerably high protein content. Previously, the protein content of insects belonging to the Orthoptera order (57-70.7%), Coleoptera (21-70.9%), Lepidoptera (31.2-57.2%) and Hymenoptera

Table 1: Chemical yield and physicochemical characterization of chitin extracted from different insect orders.

Order	Genus and species	Stage	Chitin treatment						Chitin yield (%)	DA(%)	Mw (kDa)	Reference
			Demineralization			Deproteinization						
			HCl [M]	Temp. (°C)	Time (h)	NaOH [M]	Temp. (°C)	Time (h)				
Blattodea												
	Blatella germanica	A	2	100	2	2	140	20	5	127 [a] -95[b]	nd	Kaya, Baublys, et al. (2016)
	Periplaneta americana (wings and insect body without wings)	A	4	75	2	4	150	20	Wings (18)-OP (13)	99[b]	nd	Kaya and Baran (2015)
Coleoptera												
	Agabus bipustulatus	A	1	90	1	1	110	18	14-15	117 [a]	nd	Kaya, Baran, Mentes, et al. (2014)
	Anoplotrupes stercorosus	A	2	100	2	2	140	20	20	125[a] -73[b]	nd	Kaya, Baublys, et al. (2016)
	Blaps tibialis	A	2	100	2	2	140	20	25	105[a] -110[b]	nd	Kaya, Baublys, et al. (2016)
	Calosoma rugosa	A	1	RT	-	1	100	8	5	nd	nd	Marei et al. (2016)
	Catharsius molossus	A	1.3	80 and RT	0.5+12	4	90	6	24	nd	nd	Ma et al. (2015)
	Cetonia aurata	A	2	100	2	2	140	20	18	128[a] -70[b]	nd	Kaya, Baublys, et al. (2016)
	Geotrupes stercorarius	A	2	100	2	2	140	20	20	112[a] -71[b]	nd	Kaya, Baublys, et al. (2016)
	Holotrichia parallela	A	1	100	0.5	1	80	24	15	93[a]	nd	Liu et al. (2012)
	Hydrophilus piceus	A	1	90	1	1	110	18	19-20	121[a]	nd	Kaya, Baran, Mentes, et al. (2014)
	Leptinotarsa decemlineata	A-L	2	65-75	2	2	80-90	16	A=20 and L=7	A=108[b] and L=232[b]	nd	Kaya, Baran, Erdoğan, et al. (2014)
	Melolontha melolontha	A	4	75	2	4	150	18	13-14	91[b]	nd	Kaya, Baublys, et al. (2014)
	Tenebrio molitor	A	2	50	24	2	50	24	12	Nd	894	Sáenz-Mendoza et al. (2019)
Diptera												
	Calliphora vicina	A	2	100	2	2	140	20	8	135[a] -120[b]	nd	Kaya, Baublys, et al. (2016)
Hemiptera												
	Cicada slough	A	1	100	0.3	1	80	36	37	102[b]-91[c] -97[d]	nd	Sajomsang and Gonil (2010)
	Coreus marginatus	A	2	100	2	2	140	20	15	150[a] -79[b]	nd	Kaya, Baublys, et al. (2016)
	Lygaeus equestris	A	2	100	2	2	140	20	11	161[a] -103[b]	nd	Kaya, Baublys, et al. (2016)
	Notonecta glauca	A	1	90	1	1	110	18	10-11	120 [a]	nd	Kaya, Baran, Mentes, et al. (2014)
	Pyrrhocoris apterus	A	2	100	2	2	140	20	11	123[a] -100[b]	nd	Kaya, Baublys, et al. (2016)

(Contd...)

Table 1: ***(Continued)***

Order	Genus and species	Stage	Chitin treatment						Chitin yield (%)	DA(%)	Mw (kDa)	Reference
			Demineralization			Deproteinization						
			HCl [M]	Temp. (°C)	Time (h)	NaOH [M]	Temp. (°C)	Time (h)				
	Ranatra linearis	A	1	90	1	1	110	18	15-16	133[a]	nd	Kaya, Baran, Mentes, et al. (2014)
Hymenoptera												
	Apis mellifera	A	1	RT	-	1	100	8	3	nd	nd	Marei et al. (2016)
	Bombus lapidarius	A	2	100	2	2	140	20	9	165[a] -83[b]	nd	Kaya, Baublys, et al. (2016)
	Formica clara	A	2	100	2	2	140	20	8	102[a] -111[b]	nd	Kaya, Baublys, et al. (2016)
Lepidoptera												
	Bombyx mori	C	1	100	20	1	80	24	3-4	nd	nd	Paulino et al. (2006)
	Galleria mellonella	A	2	50	24	2	50	24	6	nd	884	Sáenz-Mendoza et al. (2019)
Odonata												
	Anax imperator	L	1	90	1	1	110	18	11-12	86[a]	nd	Kaya, Baran, Mentes, et al. (2014)
	Cordulia aenea	A	2	100	2	2	140	20	10	114[a] -91[b]	nd	Kaya, Baublys, et al. (2016)
	Libellula quadrimaculata	A	2	100	2	2	140	20	10	146[a] -91[b]	nd	Kaya, Baublys, et al. (2016)
Orthoptera												
	Ailopus simulatrix	A	4	75	1	2	175	18	5	nd	5.3	Kaya, Erdogan, Mol, and Baran (2015)
	Ailopus strepens	A	4	75	1	2	175	18	7	nd	5.2	Kaya, Erdogan, et al. (2015)
	Brachystola magna	A	1	97	0.5	1	82	24	10	nd	127	Monter-Miranda et al. (2016)
	Calliptamus barbarus	A	1	100	0.5	1	80-90	21	21	88[a]	nd	Kaya, Baran, et al. (2015)
	Celes variabilis	A	4	75	2	4	150	20	7[e] -10[f]	181[b,e]-125[b,f]	nd	Kaya, Lelešius, et al. (2015)
	Decticus verrucivorus	A	4	75	2	4	150	20	10[e] -12[f]	115[b,e]-109[b,f]	nd	Kaya, Lelešius, et al. (2015)
	Dociostaurus maroccanus	A-N	2	55	1	2	50	18	A=14 and N=12	A=232[b] and N=187[b]	nd	Erdogan and Kaya (2016)
	Duroniella fracta	A	4	75	1	2	175	18	6	nd	5.9	Kaya, Erdogan, et al. (2015)
	Duroniella lacticornis	A	4	75	1	2	175	18	7	nd	5.6	Kaya, Erdogan, et al. (2015)
	Melanogryllus desertus	A	4	75	2	4	150	20	5[e] -7[f]	131[b,e]-169[b,f]	nd	Kaya, Lelešius, et al. (2015)
	Oedipoda caerulescens	A	4	75	1	2	175	18	9	nd	6.2	Kaya, Erdogan, et al. (2015)
	Oedaleus decorus	A	1	100	0.5	1	80-90	21	17	91[a]	nd	Kaya, Baran, et al. (2015)

(Contd...)

Table 1: ***(Continued)***

Order	Genus and species	Stage	Chitin treatment						Chitin yield (%)	DA(%)	Mw (kDa)	Reference
			Demineralization			Deproteinization						
			HCl [M]	Temp. (°C)	Time (h)	NaOH [M]	Temp. (°C)	Time (h)				
	Oedipoda miniata	A	4	75	1	2	175	18	8	nd	6.8	Kaya, Erdogan, et al. (2015)
	Paracyptera labiata	A	4	75	2	4	150	20	7[e] -8[f]	163[b,e]-130[b,f]	nd	Kaya, Lelešius, et al. (2015)
	Pyrgomorpha cognata	A	4	75	1	2	175	18	7	nd	5.5	Kaya, Erdogan, et al. (2015)
	Schistocerca gregaria	A	1	RT	-	1	100	8	12	nd	nd	Marei et al. (2016)

*nd = Not determined; A = Adult; L = Larvae; RT = Room temperature; OP = Insect body without wings; Mw = Molecular weight.
[a]Acetylation degree determination by infrared spectroscopy with Fourier transform.
[b]Acetylation degree by elemental analysis.
*nd = Not determined; RT = Room temperature; A = Adult; L = Larvae; C = Chrysalides; Mw = Molecular weight.
[a]Acetylation degree determination by infrared spectroscopy with Fourier transform (FTIR).
[b]Acetylation degree determination by elemental analysis.
[c]Acetylation degree determination by proton nuclear magnetic resonance spectroscopy (HNMR).
[d]Acetylation degree determination by C cross-polarization magic-angle-spinning (CP/MAS) NMR spectroscopy.
*nd = Not determinated; RT = Room temperature; A = Adult; N = Nymph; Mw = Molecular weight.
[a]Acetylation degree determination by infrared spectroscopy with Fourier transform.
[b]Acetylation degree determination by elemental analysis.
[c]Acetylation degree determination by proton nuclear magnetic resonance spectroscopy (HNMR).
[d]Acetylation degree determination by C cross-polarization magic-angle-spinning (CP/MAS) NMR spectroscopy.
[e]Female insect.
[f]Male insect.

(9.2-75.1%) has been evaluated, finding variation between among them, even among species of the same order (Ramos-Elorduy et al., 2002), without considering other variables such as development status, diet within other characteristics.

The protein content in crustaceans, such as shrimp shells (≈ 30-34%) and crabs (≈ 15-17%) seems to be lower than the content in insects (Abdou et al., 2008). For this reason, it is considered important to use an adequate method for the efficient elimination of proteins. During this stage various chemical reagents have been used, like $CaHSO_3$, $Ca(OH)_2$, Na_2CO_3, NaOH, Na_3PO_4, $NaHCO_3$, $NaHSO_3$, Na_2S, Na_2SO_3, K_2CO_3, and KOH; however, the most widely used is NaOH (Younes and Rinaudo, 2015). The evaluation of the nitrogen content in chitin, allows to determining the residual protein content in the sample. The theoretical value considered for a totally acetylated chitin is 6.89%, and a percentage above this value suggests residual protein in chitin (Majtán et al., 2007). Recent studies have obtained chitins below this value, indicating a minimal amount of residual protein. With respect to the nitrogen content, decreases have been reported after the application of the deproteinization treatment (the passage of raw material to chitin) in the insects *H. parallela* (raw material, 11.3%, chitin, 6.3%) and in the cicada sloughs (Hemiptera: Cicadidae) (raw material, 7.7%; chitin; 6.3%) (Liu et al., 2012; Sajomsang and Gonil, 2010). These values are even closer to the theoretical value than those previously reported in the crustaceans *Oniscus asellus* (Isopoda: Oniscidae) (4.8%) and shrimp (4.9%) (Majtán et al., 2007; Kaya et al., 2014).

Regarding the procedures reported in the literature for the treatment of deproteinization in insects, several methodologies can be observed, with concentrations between 1 and 4 M being reported for 8 and 36 h at temperatures ranging from 50 °C to 175 °C. In the Arachnida and Malacostraca classes concentrations of 0.5-4 M have been reported; 2-20 h; temperatures that ranged from 90 °C to 150 °C (Table 2). Commercially, the biopolymers of chitin and chitosan require that they present a white color or as close to this color; however, the chitin obtained even after undergoing demineralization and deproteinization processes shows a variability of colors according to the source of obtaining. This is because there are differences in the melanin content according to the morphological conformations of each insect species (Nemtsev et al., 2004). For example, cicada sloughs chitin showed a brown color, this indicated a residual pigment, which was eliminated when treating this chitin with the sodium hypochlorite oxidizing agent (NaClO) at 6% (Sajomsang and Gonil, 2010). Due to the above, and in order to improve the quality (emulating the commercial products) of the insect biopolymers, it is important to adjust the procedures to eliminate remaining impurities, depigment or decolorize the samples, according to the source of production. In addition to NaClO, some procedures have been reported using different chemical reagents, such as potassium permanganate,

Table 2: Chemical yield and physicochemical characterization of chitin extracted from different species of arachnids and crustaceans.

Class Order	Genus and species	Stage	Chitin treatment						Chitin yield (%)	DA(%)	Mw (kDa)	Reference
			Demineralization			Deproteinization						
			HCl [M]	Temp. (°C)	Time (h)	NaOH [M]	Temp. (°C)	Time (h)				
Arachnida												
	Argiope bruennichi	A	2	100	2	2	140	20	6	98[a] -118[b]	Nd	Kaya, Baublys, et al. (2016)
	Chaetopelma olivaceum	A	2	100	2	2	140	20	11	152[a] -10[b]	Nd	Kaya, Baublys, et al. (2016)
	Geolycosa vultuosa	A	4	60-35	1	1	130-135	16	8-9	97[b]	Nd	Kaya, Seyyar, Baran, Erdoğan, and Kar (2014)
	Hogna radiata	A	4	60-65	1	1	130-135	16	7	99[b]	Nd	Kaya, Seyyar, et al. (2014)
	Ixodes ricinus	A	2	100	2	2	140	20	6	138[a]-103[b]	Nd	Kaya, Baublys, et al. (2016)
Malacostraca												
	Penaeus aztecus shells	A	1	RT	-	1	105-110	-	22	Nd	Nd	Abdou, Nagy, and Elsabee (2008)
	Penaeus durarum shells	A	1	RT	-	1	105-110	-	24	Nd	Nd	Abdou et al. (2008)
	Crabs shells	A	1	RT	-	1	105-110	-	17	Nd	Nd	Abdou et al. (2008)
	Litopenaeus vannamei shells	A	0.55	RT	1.5	0.5	90	2	36	Nd	Nd	Vilar, Rubio, Alves, and De Campos-Takaki (2016)
	Penaeus monodon shells	A	1	RT	-	1	100	8	10	Nd	Nd	Marei et al. (2016)
	Procambarus clarkii shells	A	1	RT	-	1	105-110	-	21	Nd	Nd	Abdou et al. (2008)
	Asellus aquaticus	A	1	90	1	1	110	18	5-6	86[a]	Nd	Kaya, Baran, Mentes, et al. (2014)
	Oniscus asellus	A	4	75	2	4	150	18	6-7	169[b]	Nd	Kaya, Baublys, et al. (2014)

*nd=Not determined; A=Adult; RT=Room temperature; Mw=Molecular weight.
[a]Acetylation degree determination by infrared spectroscopy with Fourier transform.
[b]Acetylation degree determination by elemental analysis.

water-methanol-chloroform mixture (4:2:1), ethanol and acetone (Liu et al., 2012; Kaya et al., 2014; Marei et al., 2016). Hot acetic acid, cold formic acid and a mixture of ammonium sulfate/sulfuric acid, also have efficient results in the removal of pigments from crustacean exoskeletons (Tharanathan and Kittur, 2003).

It is important to mention that out of the methods reported for chitin and chitosan extraction from insect source, they have only been carried out through chemical treatments; however, chemical treatments have several drawbacks: (i) They cause damage on the physicochemical properties of chitin and lead to a decrease in MW and DA%, which affects the intrinsic properties of purified chitin; (ii) They affect the effluent of wastewater that contains some chemical products, and (iii) They increase the cost of the chitin purification processes (Younes and Rinaudo, 2015). So, the application or development of environmentally friendly techniques (using enzymes and microorganisms) for the extraction of chitin and chitosan from insect could represent a great research opportunity analogous to how they have been carried out in the extraction of these biopolymers from conventional sources (waste from the maritime industry and some fungi).

CHITOSAN OBTAINING METHODS

Chitin's insolubility (α and β forms) in regular solvents represents a great trouble for its application usage in different applications, that is why the extraction of its main derivative, chitosan, has been considered. This polysaccharide is obtained by the deacetylation of chitin,

which is subjected to a treatment with concentrated alkaline solutions and a thermal treatment at high temperature. During deacetylation, the acetyl groups are eliminated, and a depolymerization reaction occurs, also affecting the molecular weight (Younes and Rinaudo, 2015). In general, chitin can be converted into chitosan by enzymatic treatments or by chemical processes; however, regarding to what was previously reported in the production of insect chitosan, it has only been by chemical process (Table 3). Chemical methods are widely used for commercial preparation purposes. Chitin's deacetylation is carried out both, homogenously (Sannan et al., 1976) or heterogeneously (Chang et al., 1997). In this last method, chitin is treated with NaOH solution (high concentration and temperature) for a few hours. Regarding to obtaining insect chitosan, previous studies have focused on the heterogeneous method. On the subject of the procedures reported in the literature for the treatment of deacetylation of insect chitin, various conditions are observed, with NaOH concentrations between 18 M, 40%, 50% and 60% being reported during 1 and 24 h at ranging temperatures from 70 °C to 150 °C (Table 3).

Table 3: Yield and physicochemical characterization of chitosan extracted from different insect species.

Order	Genus and species	Stage				Deacetylation treatment				Reference
			Chitosan yield (%)	Mw (kDa)	DD(%)	NaOH (%)	Relation (w/v)	Time (h)	Temp. (°C)	
Coleoptera										
	Agabus bipustulatus	A	71	nd	nd	60	-	2	120	(Kaya, Baran, Mentes, et al., 2014)
	Catharsius molossus	A	72	450	95[c]	18 M[g]	-	24;7[h]	RT; 90[h]	(Ma et al., 2015)
	Hydrophilus piceus	A	74	nd	nd	60	-	2	120	(Kaya, Baran, Mentes, et al., 2014)
	Leptinotarsa decemlineata	A-L	72[e]-67[d]	3[e]-3[d]	82[b, e] and 76[b, d]	50	01:20	3	100	(Kaya, Baran, Erdoğan, et al., 2014)
	Tenebrio molitor	A	nd	254	89[c]	60	-	2	120	(Sáenz-Mendoza et al., 2019)
Diptera										
	Musca domestica	L	nd	426	90[c]	40	-	8	70	(Ai, Wang, Yang, Zhu, & Lei, 2008)
Hemiptera										
	Notonecta glauca	A	69	nd	nd	60	-	2	120	(Kaya, Baran, Mentes, et al., 2014)
	Ranatra linearis	A	70	nd	nd	60	-	2	120	(Kaya, Baran, Mentes, et al., 2014)
Lepidoptera										
	Bombyx mori	C	73-97	nd	nd	40	-	1-6	100	(Paulino et al., 2006)
	Galleria mellonella	A	nd	253	81[c]	60	-	2	120	(Sáenz-Mendoza et al., 2019)
Odonata										
	Anax imperator	A	67	nd	nd	60	-	2	120	(Kaya, Baran, Mentes, et al., 2014)
Orthoptera										
	Brachystola magna	A	81	25.8	57[c]	40	-	5.5	105-110	(Monter-Miranda et al., 2016)
	Calliptamus barbarus	A	74-75	nd	88[a]	50	01:15	2	130	(Kaya, Baran, et al., 2015)
	Dociostaurus maroccanus	A-N	82[e]-77[f]	7[e] and 6[f]	64[b,e] – 22[b,f]	60	-	4	150	(Erdogan & Kaya, 2016)
	Oedaleus decorus	A	75-76	nd	91[a]	50	01:15	2	130	(Kaya, Baran, et al., 2015)

*nd=Not determined; A=Adult; C=Chrysalide, L=Larvae, N=Nymph; Mw=Molecular weight; RT=Room temperature.
[a]Acetylation degree determination by infrared spectroscopy with Fourier transform.
[b]Acetylation degree determination by elemental analysis.
[c]Acetylation degree determination by potentiometric titration.
[d]Insect in larvae stage.
[e]Insect in adult stage.
[f]Insect in nymph stage.
[g]Alkaline solution was replaced three times during deacetylation.[h]24 h room temperature was applied then 7 h at 90°C.

CHITIN AND CHITOSAN YIELDS

The content of chitin in insect organisms range from 2.5 to 36.6% (Table 1). The highest content of chitin in the Insecta class has been recorded for the Hemiptera (10-37%), followed by Coleoptera (5-25%), Orthoptera (5-21%), Blattodea (5-18%), Odonata (10-12%), Hymenoptera (3-9%) and Diptera (8%) orders. The yields of the insects represent higher content than the species of the Arachnida class (6- 11%) and similar to the species of the Malacostraca class (5-36%) (Table 2). It can be concluded that the content of chitin can vary between species, genera, families, orders and classes of insects. Given the similarity in the content of chitin between crustaceans and insects, it can be inferred that the latter are a potential source of chitin.

In previous studies, differences have also been observed in the yield of chitin depending on the stages of development, and whether the insects are holometabolous or hemimetabolous. For example, Erdogan and Kaya (2016) evaluated nymphs and adults of the insect *Dociostaurus maroccanus* (Orthoptera: Acrididae) and found that the contents of chitin in adults (14%) and nymphs (12%) were similar, so that it can be assumed that the development of the chitin structure in the nymph is almost complete. However, in another study conducted on a holometabolous insect (*Leptinotarsa decemlineata*: Coleoptera: Chrysomelidae), they found great differences in yield, being higher in adults (20%) than in larvae (7%) (Kaya et al., 2014). This indicates that the insects have different contents of chitin in their different stages of development and stages or instars (larval and nymph) and that the content of chitin increases according to its size.

In contrast, it has been shown that the properties of chitin are affected by different segments of the insect body. Five segments of *A. mellifera* body, among them thorax, legs, abdomen, wings and head were extracted and analyzed by Kaya et al. (2015), who found higher chitin content in the legs (13.3%) compared with the other segments (6.8 to 8.9%).

Similarly, Kaya et al. (2015) evaluated four species of grasshoppers (*Celes variabilis* (Orthoptera: Acrididae), *Decticus verrucivorus* (Orthoptera: Tettigoniidae), *Melanogryllus desertus* (Orthoptera: Gryllidae), *Paracyptera labiata* (Orthoptera: Acrididae) and found a relationship between the genus (female-male) and the content of chitin, observing in addition, a higher content of chitin in males (11.8%) than in females (4.7%). As for the yield of chitosan, it varies in a range of 67-82% (Table 3). The highest content of chitosan in the Insecta class was registered for the order Lepidoptera (73-97%), followed by Orthoptera (74-82%), Coleoptera (67-74%), Hemiptera (69-70%) and, Odonata (67 %). In general, it can be observed that these insect orders showed similar yields.

CHITIN AND CHITOSAN PHYSICOCHEMICAL CHARACTERIZATION

Molecular weight (Mw)

It has been observed that two main factors that control these properties are: 1) The isolation method, and 2) The source of chitin. The evaluation of the deacetylation degree (% DD) and molecular weight (Mw) are physicochemical variables of great interest, as a result of the influence they have on the performance of the various functional properties of these biopolymers (Aranaz et al., 2009). Due to the viscosity of chitosan, the determination of the variable Mw is considered of great importance in several areas such as biochemistry and biopharmacology (Austin et al., 1981). In connection with the studies carried out in the determination of the Mw of insect chitins, these are scarce, and most have been carried out in species of the order Orthoptera; however, as a result of the importance of this variable, we consider that more studies are needed in chitins of other insect orders. Among the studies reported, Kaya et al. (2015) analyzed the chitins of seven Orthopteran species and found similar molecular weights (≈5-7 kDa), which indicated a low relation between the Mw and the source of production. As regards the Mw of chitosan, very different values were observed between the species *C. molossus* (450 kDa), *L. decemlineata* (2.7 kDa), *Musca domestica* (Diptera: Muscidae) (426 kDa), *Brachystola magna* (Orthoptera: Romaleidae) (25.8 kDa), *Galleria mellonella* (Lepidoptera: Pyraloidea) (884 kDa), *Tenebrio molitor* (Coleoptera: Tenebrionidae) (894 kDa) and *D. maroccaus* (7-6 kDa) (Kaya et al., 2014; Ma et al., 2015; Monter-Miranda et al., 2016; Erdogan and Kaya, 2016; Sáenz-Mendoza et al., 2019). The differences in these results can be attributed to the source of production, measurement methods and deacetylation treatment conditions (type of chemical reagent, concentration, time, temperature, repetition of alkaline steps, atmospheric pressure, particle size and chitin ratio-solvent, mainly) (Younes and Rinaudo, 2015).

Deacetylation degree (%DD)

The determination of the deacetylation degree (%DD) or its inverse form, known as acetylation degree (%AD, where %AD = 100-%DD), is considered as a diagnosis to classify the biopolymer as chitin or chitosan. The determination of %DD for chitosan can be carried out by different techniques: infrared spectroscopy, elemental analysis, potentiometric titration and ^{13}C-NMR (Aranaz et al., 2009; Younes and Rinaudo, 2015). In Tables 1, 2 and 3 %DD and %AD, are shown in species of the Insecta, Arachnida and

Crustacea classes; however, it is observed that the results are variable, even when evaluating the same species with different technique. The %AD (evaluation by elemental analysis) of chitin from organisms ranged from 72-232% (Table 1). The highest content of chitin in the Insecta class was registered for the order Orthoptera (109-232%), followed by Coleoptera (71-232%), Hemiptera (79-111%), Blattodea (94-99%). In the cases in which the %AD was higher than 100%, it can be attributed to an incomplete elimination of some inorganic materials of the polymer structure (Juárez-de La Rosa et al., 2012).

About the %DD of insects, Marei et al. (2016) determined (by potentiometric titration) a higher %DD (98%) in desert locust (*Schistocerca gregaria*, Orthoptera: Acrididae) than in the honey bee *A. mellifera* (96%), shrimp *Penaeus monodon* (Decapoda: Penaeidae) (74%) and the *Calosoma rugosa* beetle (Coleoptera: Carabidae) (95%). Similarly, Kumari et al. (2017) determined (through FTIR) a higher %DD in shrimp exoskeletons (78%) than in fish (75%) and crab (70%) shells. The differences between the results can be attributed to the source of production, measurement methods and to the deacetylation treatment conditions, atmospheric pressure, particle size and chitin-solvent ratio (Younes and Rinaudo, 2015). In previous researches, a relationship between the deacetylation percentage and the stage of development of the specimen has been observed. The deacetylation degree (%DD) for chitosan derived from adult potato beetles of Colorado *L. decemlineata* was 71%; while the %DD value for the chitosan of the larvae was 64%. The difference found between the species could be due to the fact that chitin is partially deacetylated in a natural way (it can vary between 5-25%, depending on the source) so that there is an adequate physiological function in the cuticle (Muthukrishnan et al., 2016).

MORPHOLOGICAL CHARACTERIZATION

Through electronic microscopy, the surface morphology of chitin and chitosan isolated from different insect sources has been studied and different forms have been reported. According to this, seven forms (types) have been identified. The first type has a porous structure and was observed in the insect *Blattella germanica* (Blattodea: Blattellidae) and *Formica clara* (Hymenoptera: Formicidae). The second type has long and weak fibers (it was observed in *Anoplotrupes stercorosus*, Coleoptera: Geotrupidae and *Blaps tibialis*, Coleoptera: Tenebrionidae). The third type consists of long, strong fibers (*Cetonia aurata*, Coleoptera: Scarabaeidae, *Calliphora vicina*, Diptera: Calliphoridae, and *Argiope bruennichi*, Araneae: Araneidae). The fourth type has a surface of weak and broken fibers of (*Geotrupes stercorarius*, Coleoptera: Geotrupidae, and *Libellula quadrimaculata*, Odonata: Libellulidae). The fifth type showed fibers and pores, and it was observed in *Coreus marginatus*; Hemiptera: Coreidae, *Lygaeus equestris*; Hemiptera: Lygaeidae, *Bombus lapidarius*; Hymenoptera: Apidae and *Cordulia aenea*; Odonata: Corduliidae. The sixth type showed fibers in the form of a mesh and large pores (observed in *Pyrrhocoris apterus*, Hemiptera: Pyrrhocoridae and *Chaetopelma olivaceum*, Araneae: Theraphosidae). The seventh type consisted of a morphology of nested complex fibers (*Ixodes ricinus*, Ixodida: Ixodidae) (Kaya et al., 2016).

However, in addition to the influence of the source of production, a relationship with the stage of development has also been observed (Ma et al., 2015; Zelencova et al., 2015; Kaya et al., 2016). The morphological aspect of these biopolymers is essential to suggest their potential applications (Jayakumar et al., 2011; Abdelmalek et al., 2017; Ahsan et al., 2017). For instance, Aranaz et al. (2009) suggested that chitins with a structure in the form of fibrils could be used in the textile industry; while chitin structures with the presence of pores are feasible for use in the formation of membranes or films for the transport of drugs and active substances (Santos et al., 2008; Bhattarai et al., 2010; Li et al., 2011).

STRUCTURAL AND THERMAL CHARACTERIZATION

Along with the crystalline structure of chitin, in nature there are three polymeric conformations; α, β and γ. The α-chitin is composed of antiparallel chains; β-chitin, of parallel chains; and γ-chitin, composed of a mixture of parallel and antiparallel chains (Greven et al., 2016). So far, all the insects that have been studied have showed α-chitin polymorphism, which is the most stable and common conformation among the exoskeletons of several organisms including crustaceans, fungi and yeasts (Younes and Rinaudo, 2015). The polymorphism of β-chitin has been reported in mollusks such as squid feathers, and the γ form, only in insect cocoons (Marei et al., 2016). Using some analytical tools such as infrared spectroscopy with Fourier transform and X-ray diffraction has confirmed the type of polymorphism of these crystalline structures. In addition, some recent studies are included in relation to the thermal characterization of these biopolymers by thermogravimetric studies.

Infrared spectroscopy by Fourier transform (FTIR)

The bands displayed in FTIR spectra show some differences between α and β polymorphism of chitin. β -chitin spectra show two bands at around wavenumber region 1560 cm^{-1} (amide II) and 1660 cm^{-1} (amide I) (Abdelmalek et al., 2017). For α-chitin, are observed three

bands and is due to the amide I band split into two bands (Hassainia et al., 2018). The bands are shown at around wavenumber region 1558, 1627 and 1660 cm^{-1} (Cárdenas et al., 2004). All the insects investigated so far shown the typical α-chitin conformation bands. Kaya et al. (2016) analyzed chitin in 13 different insect species belonging to the order Blattodea, Coleoptera, Diptera, Hemiptera, Hymenoptera and Odonata; and observed bands similar between the samples in FTIR spectra. The bands observed in spectra of different chitins samples (functional groups and vibration modes in determined wavenumber region $1/\lambda$) are shown below (position average of each band observed): ≈ 3434 cm^{-1} (O-H stretching); ≈ 3262 and ≈ 3103 cm^{-1} (N-H stretchig); ≈ 2924 cm^{-1} (CH_3 symmetric stretch and CH_2 asymmetric stretch); ≈ 2865 cm^{-1} (CH_3 symmetrical stretch); ≈ 1654 cm^{-1} (stretching of the secondary amide C=O (amide I)); ≈ 1620 cm^{-1} (stretching of the secondary amide C=O (Amide I)); ≈ 1553 cm^{-1} [N-H bending and C-N stretching (amide II)]; 1420 cm^{-1} (CH_2 bending and CH_3 deformation); ≈ 1376 cm^{-1} (CH bending and CH_3 symmetric deformation); 1308 cm^{-1} [CH_2 wagging (amide band III)]; ≈ 1258 cm^{-1} (NH bending); ≈ 1203 cm^{-1} (C-H bonds); ≈ 1154 cm^{-1} (asymmetric bridge oxygen stretching); ≈ 1113 cm^{-1} (asymmetrical in-phase ring stretching mode); ≈ 1067 cm^{-1} (C-O-C asymmetric stretch in phase ring); ≈ 1010 cm^{-1} (C-O asymmetric stretch in phase ring); ≈ 952 cm^{-1} (CH_3 wagging); ≈ 895 cm^{-1} (CH ring stretching). In other studies, in spectra of chitin from insects [*Vespa crabro* (Hymenopera: Vespidae), *Vespa orientalis* (Hymenopera: Vespidae), *Vespula germanica* (Hymenopera: Vespidae), *Brachytrupes portentosus* (Ortoptera: Gryllidae) and from commercial shrimp, were observed similar bands approximately near to previously described wavenumbers regions (Kaya et al., 2015; Ibitoye et al., 2018).

On another hand, chitosan polymer spectra show two characteristic bands at wavenumber region ≈ 1650 and1590 cm^{-1} (Chatterjee et al., 2005; Kaya et al., 2014). The insect chitosan FTIR spectra shown similar bands. For example, Kaya et al. (2014) analyzed five different aquatic insect [*Agabus bipustulatus* (Coleoptera: Dytiscidae), *Anax imperator* (Odonata: Aeshnidae), *Hydrophilus piceus* (Coleoptera: Hydrophilidae), *Notonecta glauca* (Hemiptera: Notonectidae), *Ranatra linearis* (Hemiptera: Nepidae) and observed bands similar between the samples. The bands observed in spectra of different chitosan samples are shown below (position average of each band observed): ≈ 3343 cm^{-1} [ν(NH_2) assoc. in primary amines and ν (OH) assoc. in pyranose ring]; ≈ 2918 cm^{-1} [ν_{as}(CH_2) in CH_2OH group)]; ≈ 2856 cm^{-1} [ν (C-H) in pyranose ring]; ≈ 1654 cm^{-1} [ν (C=O) in $NHCOCH_3$ group (Amide I)]; ≈ 1590 cm^{-1} [ν (NH_2) in $NHCOCH_3$ group (Amide II)]; 1420 cm^{-1} [δ(CH_2) in CH_2OH group]; ≈ 1375 cm^{-1} [δ_s(CH_3) in $NHCOCH_3$ group]; 1320 cm^{-1} [δ (C-H) in pyranose ring]; ≈ 1258 cm-1[Complex vibrations of NHCO group (Amide III)]; ≈ 1149 cm^{-1} [ν_s(C-O-C) (glycosidic linkage)]; ≈ 1058 cm^{-1} [ν_{as}(C-O-C) (glycosidic linkage)]; ≈ 1022 cm^{-1} [ν (C-O) in secondary OH group]; ≈ 990 cm^{-1} [ν (C-O) in primary OH group]; ≈ 892 cm^{-1} (pyronose ring skeletal vibrations). According to the above, the same bands have been observed in chitosans isolated from the insects *Calliptamus barbarus* (Orthoptera: Acrididae), *Oedaleus decorus* (Orthoptera: Acrididae); *Brachytrupes portentosus* (Ortoptera: Gryllidae) and in addition, similar bands have been showed in commercial chitosans (Kaya et al., 2015; Ibitoye et al., 2018). This technique allows to know the effectiveness of the used methods for the isolation and purification of chitin and chitosan.

X-Ray diffraction (XDR)

Chitin and chitosan biopolymers crystallinity degree or index evaluation is important to identify its possible application in various areas (Aranaz et al., 2009; Sáenz-Mendoza et al., 2019). In general, the XRD peaks of chitins and chitosans isolated from the different insect species were similar with crustacean sources. By analyzing XRD the α-chitins showed six peaks, which include two pronounced peaks and four weak peaks; these peaks occurred around 2θ = 9.56, 12.76, 19.72, 21.12, 23.96 and 26.64°; while in the samples of chitosan only two peaks of greater intensity have been observed, around 2θ = 10.76 and 20.30°.In the three types of polymorphism (α, β, γ), the most pronounced peaks of crystallinity are located close to 2θ = 9-10° and 19-20°, the second being the highest intensity, both for chitin and chitosan (Focher et al., 1992; Kaya et al., 2015).

According to insect chitins and chitosans crystallinity index, variations were observed between the Insecta class (chitin = 54-91%, chitosan = 49-83%) and Malacostraca (chitin = 57-77%; chitosan = 36-66%) (Fig. 2). Regarding to the variability in the crystallinity index between the chitins of different insect orders, a high value was observed for the Coleoptera order (54-91%) followed by, Blattodea (70-87%), Hemiptera (60-87%), Orthoptera (63-80%), Hymenoptera (70-76%), Odonata (64-77%) and Diptera (67%); while in chitosan, the highest value was registered for the Hemiptera (83%), followed by Orthoptera (69%), Lepidoptera (66%), Coleoptera (49-64%), and Hymenoptera (59%), (Figure 2).The differences between the classes and orders can be attributed to the structures of the insects themselves and to the conditions of the deacetylation process. In relation to the previous reports on the crystallinity indexes in chitin samples, a direct relationship between a high crystallinity index and a higher hardness in the chitins of the insect exoskeletons has been suggested (Kaya et al., 2016).

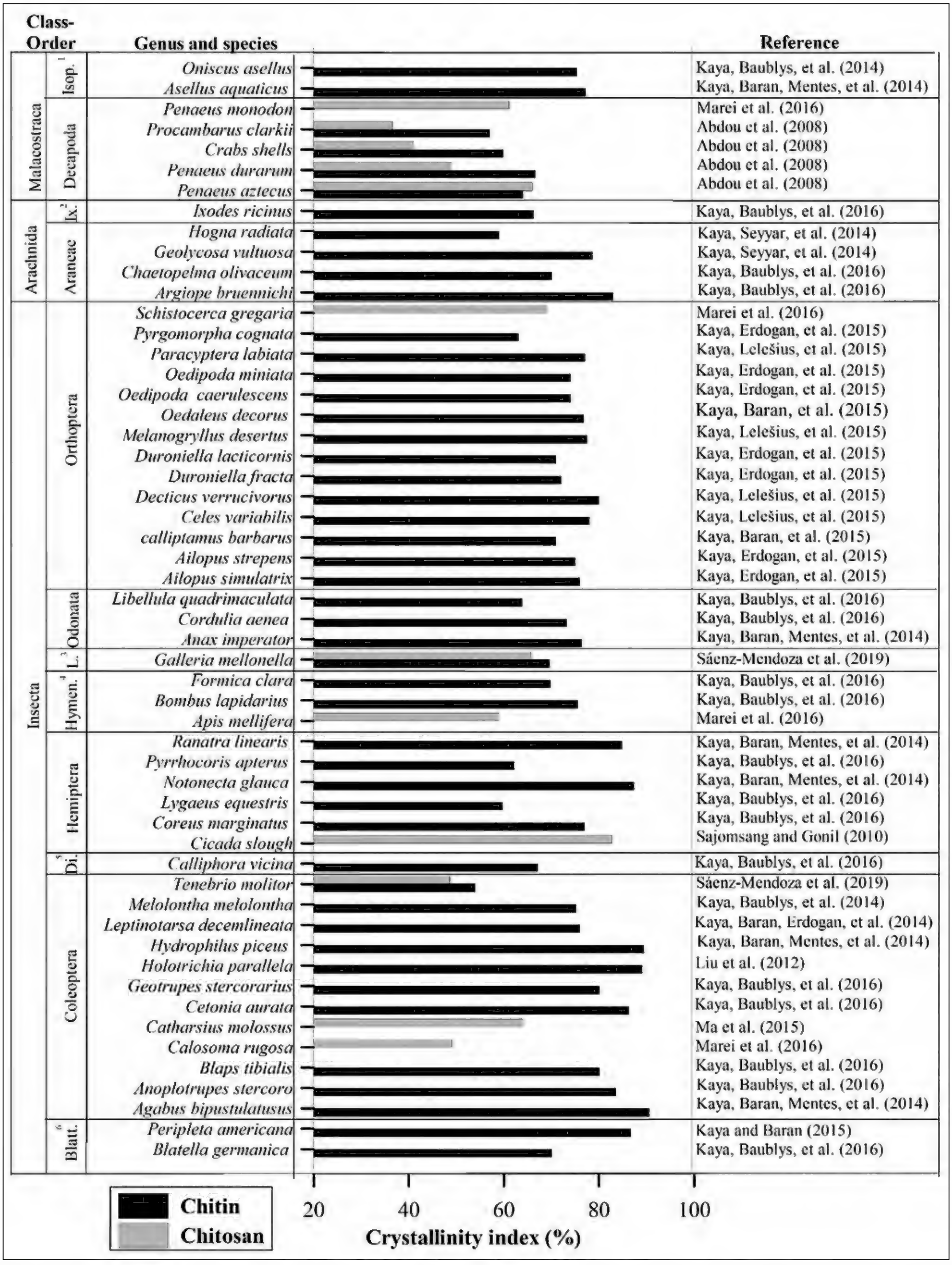

Fig 2. Crystallinity index percentage in chitin and chitosan extracted form species of the Insecta, Arachnida and Malacostraca class. [1]Isopoda; [2]Lepidoptera; [3]Hymenoptera; [4]Diptera; [5]Blattodea and [5]Ixodida.

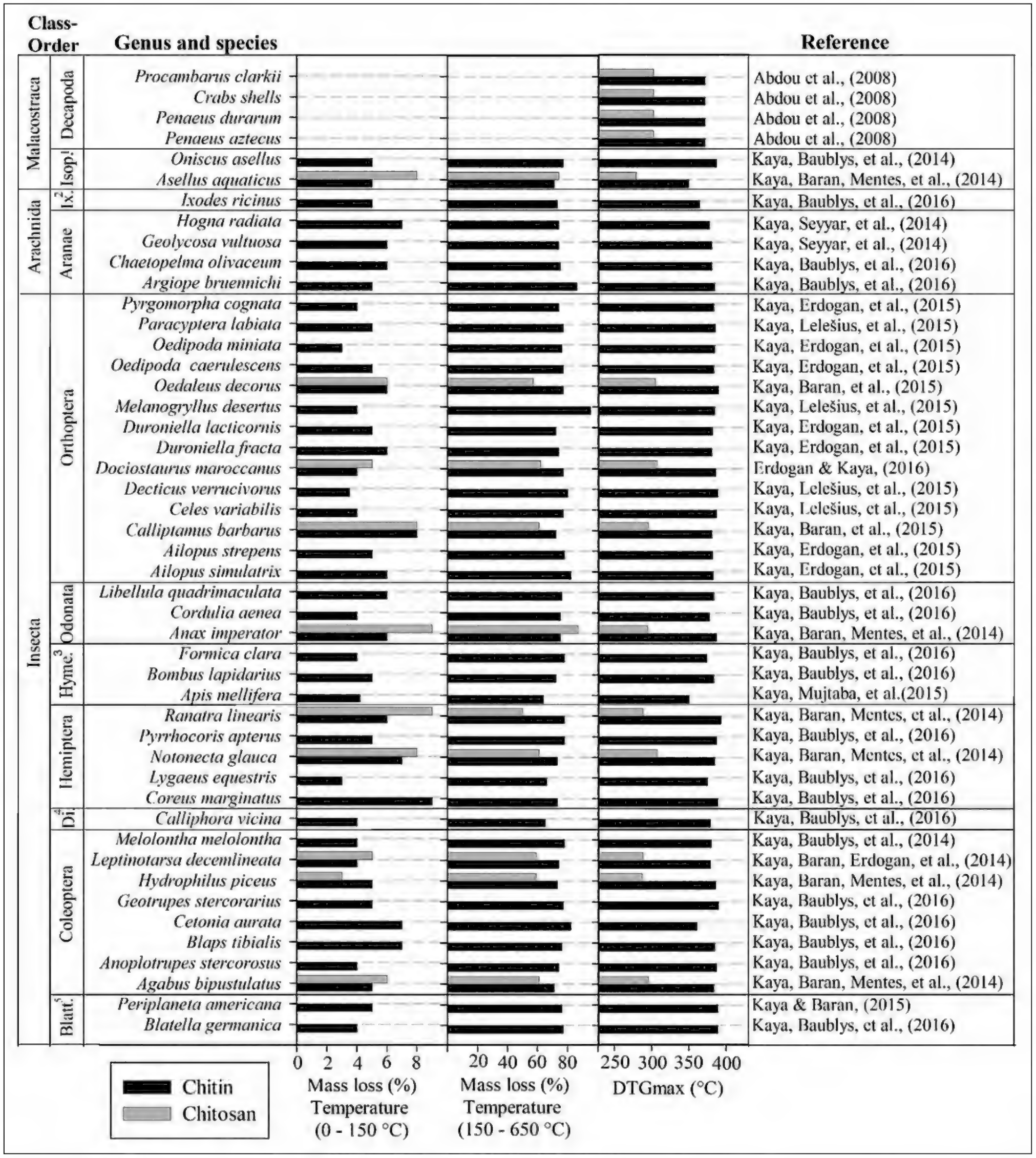
Class-Order
Genus and species
Reference
Malacostraca
Decapoda
Procambarus clarkii — Abdou et al., (2008)
Crabs shells — Abdou et al., (2008)
Penaeus durarum — Abdou et al., (2008)
Penaeus aztecus — Abdou et al., (2008)
Isop.[1]
Oniscus asellus — Kaya, Baublys, et al., (2014)
Asellus aquaticus — Kaya, Baran, Mentes, et al., (2014)
Arachnida
Ix.[2]
Ixodes ricinus — Kaya, Baublys, et al., (2016)
Aranae
Hogna radiata — Kaya, Seyyar, et al., (2014)
Geolycosa vultuosa — Kaya, Seyyar, et al., (2014)
Chaetopelma olivaceum — Kaya, Baublys, et al., (2016)
Argiope bruennichi — Kaya, Baublys, et al., (2016)
Insecta
Orthoptera
Pyrgomorpha cognata — Kaya, Erdogan, et al., (2015)
Paracyptera labiata — Kaya, Lelešius, et al., (2015)
Oedipoda miniata — Kaya, Erdogan, et al., (2015)
Oedipoda caerulescens — Kaya, Erdogan, et al., (2015)
Oedaleus decorus — Kaya, Baran, et al., (2015)
Melanogryllus desertus — Kaya, Lelešius, et al., (2015)
Duroniella lacticornis — Kaya, Erdogan, et al., (2015)
Duroniella fracta — Kaya, Erdogan, et al., (2015)
Dociostaurus maroccanus — Erdogan & Kaya, (2016)
Decticus verrucivorus — Kaya, Lelešius, et al., (2015)
Celes variabilis — Kaya, Lelešius, et al., (2015)
Calliptamus barbarus — Kaya, Baran, et al., (2015)
Ailopus strepens — Kaya, Erdogan, et al., (2015)
Ailopus simulatrix — Kaya, Erdogan, et al., (2015)
Odonata
Libellula quadrimaculata — Kaya, Baublys, et al., (2016)
Cordulia aenea — Kaya, Baublys, et al., (2016)
Anax imperator — Kaya, Baran, Mentes, et al., (2014)
Hyme.[3]
Formica clara — Kaya, Baublys, et al., (2016)
Bombus lapidarius — Kaya, Baublys, et al., (2016)
Apis mellifera — Kaya, Mujtaba, et al.(2015)
Hemiptera
Ranatra linearis — Kaya, Baran, Mentes, et al., (2014)
Pyrrhocoris apterus — Kaya, Baublys, et al., (2016)
Notonecta glauca — Kaya, Baran, Mentes, et al., (2014)
Lygaeus equestris — Kaya, Baublys, et al., (2016)
Coreus marginatus — Kaya, Baublys, et al., (2016)
Di.[4]
Calliphora vicina — Kaya, Baublys, et al., (2016)
Coleoptera
Melolontha melolontha — Kaya, Baublys, et al., (2014)
Leptinotarsa decemlineata — Kaya, Baran, Erdogan, et al., (2014)
Hydrophilus piceus — Kaya, Baran, Mentes, et al., (2014)
Geotrupes stercorarius — Kaya, Baublys, et al., (2016)
Cetonia aurata — Kaya, Baublys, et al., (2016)
Blaps tibialis — Kaya, Baublys, et al., (2016)
Anoplotrupes stercorosus — Kaya, Baublys, et al., (2016)
Agabus bipustulatus — Kaya, Baran, Mentes, et al., (2014)
Blatt.[5]
Periplaneta americana — Kaya & Baran, (2015)
Blatella germanica — Kaya, Baublys, et al., (2016)
0 2 4 6 8
Mass loss (%)
Temperature
(0 - 150 °C)
20 40 60 80
Mass loss (%)
Temperature
(150 - 650 °C)
250 300 350 400
DTGmax (°C)
Chitin
Chitosan

Fig 3. Thermogravimetric analysis of chitin and chitosan extracted from species of the Insecta, Arachnida, and Malacostraca classes. [1]Isopoda; [2]Ixodida; [3]Hymenoptera; [4]Diptera; [5]Blattodea.

Thermogravimetric analysis (TGA)

Analyzes of thermal properties are necessary to evaluate the feasible applications of chitin and chitosan (Mushi et al., 2014). The thermogravimetric analysis allows to determine the mechanism of degradation and predicts the thermal stability in both biopolymers (chitin and chitosan). Most of the reported studies about insect chitin and chitosan analyzed so far, have exposed these biopolymers to a temperature interval from 0 up to 650 °C. In general, chitin's and chitosan's calorimetric curves have in common the presence of a two decomposition phases. Fig. 3 shows the maximum peak DTG (maximum decomposition temperature) and the mass

losses subjected to different temperatures (0-150 °C and 150-650 °C) in chitins and chitosan's isolated from species of the Insecta, Malacostraca and Arachnida classes. The first mass losses in chitins and chitosan were similar in the Insecta class (chitin = 3-9%, chitosan = 3-9%), Arachnida (chitin = 5-7%) and Malacostraca (chitin = 5%, chitosan = 8%), these results can be explained by the evaporation of the water molecules present in the samples.

In the second phase, the mass losses were variable between the Insecta class (chitin = 64-95%, chitosan = 50-87%), Arachnida (chitin = 73-86%) and Malacostraca (chitin = 71-77%, chitosan = 74%). This loss of mass is due to degradation of the acetylated and deacetylated units of the polymers structure (Jayakumar et al., 2009; Soon et al., 2018). Regarding to the maximum DTG, the values varied between the chitins and chitosan obtained from the Insecta class (chitin = 351-393 °C, chitosan = 288-308 °C), Arachnida (chitin = 365-385 °C) and Malacostraca (chitin = 350-387 °C, chitosan = 280-303 °C). In general, greater maximum DTG can be observed in chitins compared to chitosan. This is because chitin has a higher crystallinity (Rodríguez et al., 2010).

Recently, Kaya et al. (2014) observed that the thermal stability of chitin varied according to the stage of development of the insect, observing a greater value in adults of the Coleoptera *L. decemlineata* (DTG max: 379 °C) in comparison with the larvae (DTG max: 307 °C), inferring that the thermal stability can be explained by the higher degree of cross-linking, which in turn, depended on the greater degree of sclerotization in the cuticle of adults. Conversely, in hemimetabolous insects such as *D. maroccanus*, they found similar values between adult chitins (386 °C) and nymphs (383 °C) (Erdogan and Kaya, 2016). These results indicate that there is a research potential in relation to these issues to be able to discern and explain these feasible differences and similarities.

CONCLUSIONS

The physicochemical, morphological and structural characteristics of insect chitin and chitosan are influenced by the attributes of each insect species, such as the stage of development, the type of metamorphosis, genus (female and male) and even, the part from which these biopolymers are extracted; however, they generally have similar properties to the Malacostraca (crustaceans) and Arachnida (arachnids) classes, and are comparable with commercial samples. An advantage that predominates in insects, unlike crustaceans, is the low content of inorganic materials in the raw material, so it is inferred that moderate conditions can be used in acid treatments (compared to crustaceans), this would allow adjust the treatment according to the insect raw material, which would be feasible to apply demineralization treatments at lower cost. Due to the properties (physicochemical, morphological and structural) showed by insect chitins and chitosan's, it is concluded that insects can be valued as a potential source of supply of these biopolymers; in addition to this, it is recommended to carry out more detailed and systematic studies, which allow to evaluate the economic and ecological impact, and in this way consider the profitable exploitation of insects as sustainable and viable sources for the supply of chitin and chitosan.

FUTURE TRENDS

Although the chitin/chitosan biopolymers can be used in various areas such as biomedicine, agriculture and the food industry for its biological and functional properties (such as its antimicrobial, antioxidant, hypocholesterolemic and immunogenic properties, as well as its application in healing, training, and its capacity of films and edible coatings, among others), few studies have focused on the characterization of these biopolymers of insect origin (because most have been made in chitin's and chitosan's from commercial sources such as shrimp and crab), and knowing that the properties of chitin/chitosan depend to a great extent on the conditions and the source of production, for this reason it is considered necessary to carry out future investigations to know the properties of insect chitin/chitosan. Under this perspective, currently there are few researches where the antioxidant and antimicrobial activity are evaluated on solutions formed from chitosan, reaching favorable results; however, there is still a great potential for further research, in order to get to explore the various properties of insect chitin/chitosan.

On the other hand, we consider that in order to potentiate the use or application of insect chitosan, studies of chitosan in its various forms of presentation such as fibers, gels, sponges, microspheres, nanospheres, hydrogels and films are also necessary. The study of insect chitosan in the form of an edible film for its application in food could be an interesting research opportunity area, since food security is currently of global concern. Therefore, the use of insect chitosan in the form of a film could be a viable alternative as a packaging material for the preservation of the quality of food products.

Conflicts of interest

The authors declare no conflicts of interest.

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Author contributions

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ABBREVIATIONS

Mw, molecular weight; DD, degree of deacetylation; DA, degree of acetylation; FTIR, infrared spectroscopy with Fourier transform; XDR, x-ray diffraction; TGA, thermogravimetric analysis; DTG max, maximum decomposition temperature.

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